Name: Veerendra Kalyan Jagannadh

SR.No: 01-02-00-10-12-13-1-10479

Registered for: Ph.D

Department: Instrumentation and Applied Physics

Supervisor: Sai Siva Gorthi

Thesis Title: "Point-of-Care High-throughput Optofluidic Microscope for Quantitative Imaging Cytometry"

Synopsis

Biological research and Clinical Diagnostics heavily rely on Optical Microscopy for analyzing properties of cells. The experimental protocol for conducting a microscopy based diagnostic test consists of several manual steps, like sample extraction, slide preparation and inspection. Recent advances in optical microscopy have predominantly focused on resolution enhancement. Whereas, the aspect of automating the manual steps and enhancing imaging throughput were relatively less explored. Cost-effective automation of clinical microscopy would potentially enable the creation of diagnostic devices with a wide range of medical and biological applications. Further, automation plays an important role in enabling diagnostic testing in resource-limited settings.

This thesis presents a novel optofluidics based approach for automation of clinical diagnostic microscopy. A system-level integrated optofluidic architecture, which enables the automation of overall diagnostic work-flow has been proposed. Based on the proposed architecture, three different prototypes, which can enable point-of-care (POC) imaging cytometry have been developed. The characterization of these prototypes has been performed. Following which, the applicability of the platform for usage in diagnostic testing has been validated. The prototypes were used to demonstrate applications like Cell Viability Assay, Red Blood Cell Counting, Diagnosis of Malaria and Spherocytosis.

An important performance metric of the device is the throughput (number of cells imaged per second). A novel microfluidic channel design, capable of enabling imaging throughputs of about 2000 cells per second has been incorporated into the instrument. Further, material properties of the sample handling component (microfluidic device) determine several functional aspects of the instrument. Ultrafast-laser inscription (ULI) based glass microfluidic devices have been identified and tested as viable alternatives to Polydimethylsiloxane (PDMS) based microfluidic chips. Cellular imaging with POC platforms has thus far been limited to acquisition of 2D morphology. To potentially enable 3D cellular imaging with POC platforms, a novel slanted channel microfluidic chip design has been proposed. The proposed design has been experimentally validated by performing 3D imaging of fluorescent microspheres and cells. It is envisaged that the proposed innovation would aid to the current efforts towards implementing good quality health-care in rural scenarios. The thesis is organized in the following manner :

The overall thesis can be divided into two parts. The first part (chapters 2, 3) of the thesis deals with the optical aspects of the proposed Optofluidic instrument (development, characterization and validations demonstrating its use

in poc diagnostic applications). The second part (chapters 4,5,6) of the thesis details the microfluidic sample handling aspects implemented with the help of custom fabricated microfludic devices, the integration of the prototype, functional framework of the device.

Chapter 2 introduces the proposed optofluidic architecture for implementing the POC tool. Further, it details the first implementation of the proposed platform, based on the philosophy of adapting ubiquitously available electronic imaging devices to perform cellular diagnostic testing. The characterization of the developed prototypes is also detailed.

Chapter 3 details the development of a stand-alone prototype based on the proposed architecture using inexpensive off-the-shelf, low frame-rate image sensors. The characterization of the developed prototype and its performance evaluation for application in malaria diagnostic testing are also presented. The chapter concludes with a comparative evaluation of the developed prototypes, so far.

Chapter 4 presents a novel microfludic channel design, which enables the enhancement of imaging throughput, even while employing an inexpensive low frame-rate imaging modules. The design takes advantage of radial arrangement of microfludic channels for enhancing the achievable imaging throughput. The fabrication of the device and characterization of achievable throughputs is presented. The stand-alone optofluidic imaging system was then integrated into a single functional unit, with the proposed microfluidic channel design, a viscoelastic effect based microfluidic mixer and a suction-based microfluidic pumping mechanism.

Chapter 5 brings into picture the aspect of the material used to fabricate the sample handling unit, the robustness of which determines certain functional aspects of the device. An investigative study on the applicability of glass microfluidic devices, fabricated using ultra-fast laser inscription in the context of the microfluidics based imaging flow cytometry is presented. As detailed in the introduction, imaging in poc platforms, has thus far been limited to acquisition of 2D images. The design and implementation of a novel slanted channel microfluidic chip, which can potentially enable 3D imaging with simplistic optical imaging systems (such as the one reported in the earlier chapters of this thesis) is detailed. A example application of the proposed microfludic chip architecture for imaging 3D fluorescence imaging of cells in flow is presented.

Chapter 6 introduces a diagnostic assessment framework for the use of the developed ofm in an actual clinical diagnostic scenario. The chapter presents the use of computational signatures (extracted from cell images) to be employed for cell recognition, as part of the proposed framework. The experimental results obtained while employing the framework to identify cells from three different leukemia cell lines have been presented in this chapter.

Chapter 7 summarizes the contributions reported in this thesis. Potential future scope of the work is also detailed.

List of Publications

Patents Filed

1. *AN AUTOMATED PORTABLE MICROFLUIDIC MICROSCOPY SYSTEM AND A METHOD THEREOF* S. S. Gorthi, **V. K. Jagannadh**
(International Patent Application No: PCT/IB2015/053581, Indian Patent Application No: 2432/CHE/2014).

2. *A MICROFLUIDIC CARTRIDGE* S. S. Gorthi, **V. K. Jagannadh**, V. Guptha
(Indian Patent, Application No. 4229/CHE/2015.).

Journal Publications (as part of thesis)

9. **V. K. Jagannadh**, M. D. Mackenzie, P. Pal, A. K. Kar, and S. S. Gorthi, "Slanted channel microfluidic chip for 3D fluorescence imaging of cells in flow," **Optics Express** Vol.**24**, No. 19, (2016); (pp. 22144-22158).

8. **V. K. Jagannadh**, B. P. Bhat, L. A. N. Julius and S. S. Gorthi, "High-throughput miniaturized microfluidic microscopy with radially parallelized channel geometry," **Analytical and Bioanalytical Chemistry** Vol.**408**, No. 7, (2016); (pp. 1909-1916).

7. **V. K. Jagannadh**, G. Gopakumar, G. R. K. S. Subrahmanyam, and S.S.Gorthi, "Microfluidic microscopy-assisted label-free approach for cancer screening: automated microfluidic cytology for cancer screening," **Medical, Biological, Engineering and Computing**. (Published online) (2016);.

6. L.A.Nirupa Julius, **V. K. Jagannadh**, I. J. Michael, R. Srinivasan and S.S.Gorthi, "Design and Validation of On-chip Planar Mixer based on Advection and Viscoelastic Effects," BioChip Journal Vol.**10**, No. 1, (2016); (pp. 16-24).

5. **V. K. Jagannadh**, R.S. Murthy, R.Srinivasan and S.S. Gorthi, "Field-Portable Microfluidics-Based Imaging Flow Cytometer," **IEEE Journal of Lightwave Technology**, Vol. **33**, No. 16, 3469-3474, (2015).

4. **V. K. Jagannadh**, R.S. Murthy, R.Srinivasan and S.S. Gorthi, "Automated quantitative cytological analysis using portable microfluidic microscopy" **Journal of Biophotonics** Vol.**9**, No. 6, (2016); (pp. 586-595) (2016).

3. **V. K. Jagannadh**, J.V. Adhikari, and S.S. Gorthi, "Automated cell viability assessment using a microfluidics based portable imaging flow analyzer," AIP - Biomicrofluidics, Vol.**9**, Issue 2, 024123, (2015).

2. **V. K. Jagannadh**, Mackenzie, M.D., Pal, P., Kar, A.K and S.S. Gorthi, "Imaging Flow Cytometry with Femtosecond Laser Micromachined Glass Microfluidic Channels," **IEEE Journal of Selected topics in Quantum Electronics** Vol.**21**, No. 4, (2015); (6990492).

1. **V. K. Jagannadh**, R.Srinivasan and S.S. Gorthi, "A semi-automated, field-portable microscopy platform for clinical diagnostic applications," AIP Advances, Vol.**5**, Issue 8, 084902 (2015).

Journal Publications (as co-author)

5. S. Murali, J. V. Adhikari, **V. K. Jagannadh** and S. S. Gorthi, "Computational Approach based Cost-effective Portable Slide Scanner,"Medical Engineering and Physics (Under Review).

4. E. Banoth, **V. K. Jagannadh** and S.S.Gorthi, "Single-cell Transmittance Measurements on Blood-Smear for the Detection of Malaria ," Technology letters; Vol.**1**, No.9(2014), 29-33,ISSN:2348-8131.

3. E. Banoth, **V. K. Jagannadh** and S.S.Gorthi, "Single-Cell Optical Absorbance Characterization With High-Throughput Microfluidic Microscopy," **IEEE Journal of Selected topics in Quantum Electronics** Vol.**22**, No. 3, (2015); (6800106).

2. G. Gopakumar, **V. K. Jagannadh** and S.S.Gorthi, G. R. K. S. Subrahmanyam, "Framework for Morphometric Classification of Cells in Imaging Flow Cytometry," **Journal of Microscopy** Vol.**261**, No. 3, (2016); (pp. 307-319).

1. E. Banoth, V.K. Kasula,**V. K. Jagannadh** and S.S.Gorthi, "Optofluidic single-cell absorption flow analyzer for point-of-care diagnosis of malaria.," **Journal of Biophotonics** Vol.**9**, No. 6, (2016); (pp. 610-618) (2016).

Refereed Conference Proceedings

3. **V. K. Jagannadh**, Mackenzie, M.D., Pal, P., Kar, A.K and S.S. Gorthi, "Optofluidic Microscopy Using Femtosecond Micromachined Glass Microfluidics," OSA Technical Digest, Paper:T3A.6, Optical Society of America, (PHOTONICS 2014), Dec 13-16, 2014, Kharagpur, India.

2. E. Banoth, **V. K. Jagannadh** and S.S.Gorthi, "Single Cell Optical Transmittance Based Label-free Malaria Detection at the Point-of-care ," OSA Technical Digest, Paper:T3A.5, Optical Society of America, (PHOTONICS 2014), Dec 13-16, 2014, Kharagpur, India.

1. **V. K. Jagannadh**, R.Srinivasan and S.S. Gorthi, "A semi-automated, field-portable microscopy platform for clinical diagnostic applications," Proc. of AIP; International Conference on Light (Optics '14), March 19-21, 2014, Calicut, India.

Conference Presentations (Abstracts)

4. S.M. Modumudi, **V. K. Jagannadh**, and S.S. Gorthi, "Floating Droplet Based Polymer Lens Fabrication for Applications in Mobile Phone Microscopy," SPIE International Conference on Optics & Photonics (IOP15) 2015.

3. **V. K. Jagannadh**, R.Srinivasan and S.S. Gorthi, "An automated, field-portable microscopy platform for clinical diagnostic applications," SELECT-BIO Microfluidics and Lab-on-a-Chip India (2015).

2. E. Banoth, **V. K. Jagannadh**, R.Srinivasan and S.S. Gorthi, "Single-cell Transmittance Measurements on Blood-Smear for the Detection of Malaria ," Optics'14 International Conference on Light, Calicut, India, 2014.

1. **V. K. Jagannadh**, S.Narendranath and S.S. Gorthi, "Cellphone based Optofluidic Microscope for Space Applications ," 30th Annual In-house Symposium (ISRO-STC (Space Technology Cell)), Bangalore, India, 2013

Honors/Awards

- Worked as the student lead for an inter-disciplinary project, which went on to win the prestigious Gandhian Young Technological Innovation (GYTI) Award, 2016 (cash of Rs. 15,00,000/- ($\sim$ 22539 $))
- V.K. Jagannadh was Awarded Full Scholarship for participating in the UK-India Antimicrobiral Drug Resistance Diagnostics (AMR DxC) School, held at the University of Edinburgh.
- IEEE Bangalore Section Patent award, 2014
- IEEE Bangalore Section Publication award, 2014

Compiled on: December 28, 2016